



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US97/07700 <b>(22) International Filing Date:</b> 5 May 1997 (05.05.97)  <b>(30) Priority Data:</b> 08/643,219 3 May 1996 (03.05.96) US Not furnished 3 April 1997 (03.04.97) US  <b>(71) Applicant:</b> ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).  <b>(72) Inventors:</b> DAVIDSON, Donald, J.; 4835 Kingsway West, Gurnee, IL 60031 (US). WANG, Jieyi; 5338 Mahogany Court, Gurnee, IL 60031 (US). GUBBINS, Earl, J.; 15646 W. Birchwood Lane, Libertyville, IL 60048 (US).  <b>(74) Agents:</b> STEELE, Gregory, W. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).		<b>(81) Designated States:</b> AU, BR, CA, CN, CZ, HU, IL, JP, KR, MX, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> NOVEL ANTIANGIOGENIC PEPTIDES, POLYPEPTIDES ENCODING SAME AND METHODS FOR INHIBITING ANGIOGENESIS  <b>(57) Abstract</b>  Mammalian kringle (5) fragments and kringle (5) fusion proteins are disclosed as compounds for treating angiogenic diseases. Methods and compositions for inhibiting angiogenic diseases are also disclosed.		

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5'-AGCGGCGACGACGACGACAAG-3' (Ek-Cut-5p, SEQ ID NO:31) and  
5'-CTTGTCGTCGTCGTCGCCGCT-3' (Ek-Cut-3p, SEQ ID NO:32 coding for an  
Enterokinase cleavage site) in 40 µL of BRL ligase and ligase buffer. An enterokinase site -  
mature stromelysin (Ek-Stromelysin) PCR fragment was generated using 1 µL of this  
ligation as a template, primers SEQ ID NO:29 and kinased SEQ ID NO:31, Ulma DNA  
polymerase and buffer at 94°C, 2 mins.; then 10 cycles of 94°C, 1 min.; 44°C, 1 min.;  
72°C, 2 mins., then 72° for 7 mins.. The Ek-Stromelysin PCR fragment was gel purified.

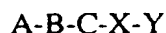
The T7-ubiquitin and Ek-stromelysin PCR fragments were ligated together in BRL  
ligase and ligase buffer. A T7-ubiquitin-Ek-stromelysin PCR fragment was then generated  
using the ligation as template and Ulma DNA polymerase and the primers SEQ ID NO:28  
and SEQ ID NO:29 at 94°C, 2' then 25 cycles of 94°C, 30 sec.; 42°C, 1 min.; 72°C, 6  
mins., then 72°C for 7 mins.

A PCR fragment was generated using the stromelysin-pET3b plasmid template with  
the primers SEQ ID NO:26 and SEQ ID NO:30 with KlenTaq (AB Peptides, St. Louis,  
MO) and *pfu* DNA polymerases at 94°C, 2' then 15 cycles of 94°C, 30 sec.; 42°C, 2 mins.;  
68°C, 20 mins.. This PCR fragment was mixed with the T7-Ubiquitin-Ek-Stromelysin  
PCR fragment and transformed into BRL DH5α maximum efficiency competent cells.  
Correct clones were identified by isolation of plasmid DNA, transfection into BL21(DE3),  
and expression studied as described above.

A PCR fragment for Ubiquitin-Ek was generated from a correct T7-Ubiquitin-Ek-  
Stromelysin expression plasmid with the primers SEQ ID NO:24 and SEQ ID NO:32 and  
*pfu* DNA polymerase at 94°C, 2' then 20 cycles of 94°C, 30 sec.; 40°C, 1 min.; 72°C, 3  
mins., 72°C, 7 mins.. The fragment was purified over a Pharmacia S-400 HR Spin column  
and ligated to the VBC1 cassette using the Rapid DNA Ligation kit. A PCR fragment was  
generated using the ligation as template and the primers SEQ ID NO:24 and 5'-  
TGAAGAGCAAAAAAAGCCCG-3' (SEQ ID NO:33) and *pfu* DNA polymerase at 94°C, 2  
mins. then 20 cycles of 94°C, 30 sec.; 40°C, 1 min.; 72°C, 2 mins., 72°C, 7 mins.. The  
PCR fragment was kinased and ligated to Upet-H prepared for blunt, phosphatased cloning.  
The ligation was transformed into competent cells and colonies were screened by PCR as  
above. Plasmid DNA was sequenced to identify correct clones of UpET-Ubi.

## WHAT IS CLAIMED IS:

1. A compound having the formula



(I)

or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl, 3-l-tyrosyl or phenylalanyl;

R<sup>4</sup> is aspartyl; and

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1 and homologues and analogues thereof.

2. The compound of Claim 1 wherein B is present and A, C, X and Y are as defined therein.

3. The compound of Claim 2 wherein X is present and A, B, C and Y are as defined therein.

4. The compound of Claim 3 wherein A and Y are present and B, C and X are as defined therein.

5. The compound of Claim 3 wherein A and Y are as defined therein and B-C-X is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;
- (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;
- (g) the sequence from amino acid positions 449-546 of SEQ ID NO:1;

- 10 (h) the sequence from amino acid positions 454-546 of SEQ ID NO:1;  
(i) the sequence from amino acid positions 525-535 of SEQ ID NO:1;  
(j) the sequence from amino acid positions 529-535 of SEQ ID NO:1; and  
(k) the sequence from amino acid positions 530-535 of SEQ ID NO:1.
6. The compound of Claim 5 wherein A is N-Ac and Y is -NH<sub>2</sub>.
7. The compound of Claim 1 wherein X is absent and A, B, C and Y are as defined therein.
8. The compound of Claim 7 wherein X, A and Y are as defined therein and B-C is the sequence from amino acid positions 529-534 of SEQ ID NO:1.
9. The compound of Claim 1 wherein B and X are absent and A, C and Y are as defined therein.
10. The compound of Claim 9 wherein C is the sequence from amino acid positions 531-534 of SEQ ID NO:1.
11. The compound of Claim 1 wherein said compound has a molecular weight of between 0.5 and 25,000 kilodaltons as determined by reducing polyacrylamide gel electrophoresis or mass spectrometry analysis and an amino acid sequence substantially similar to the corresponding amino acid sequence of SEQ ID NO: 1.
12. The compound of Claim 1 having an endothelial cell migration inhibition ED<sub>50</sub> of about 100 to about 500 pM.
13. The compound of Claim 1 having an endothelial cell proliferation inhibition ED<sub>50</sub> of about 100 to about 500 pM.
14. A compound having the formula
- $$A-B_1-C_1-X_1-Y$$
- (II)
- or a pharmaceutically acceptable salt, ester or prodrug thereof wherein
- 5 A is absent or a nitrogen protecting group;  
Y is absent or a carboxylic acid protecting group;

B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

10 C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID NO:1 and homologues and analogues thereof.

15

15. The compound of Claim 14 wherein B<sub>1</sub> and X<sub>1</sub> are absent and A, C<sub>1</sub> and Y are as defined therein.

16. The compound of Claim 8 or 10 or 15 wherein A is N-Ac and Y is -NH<sub>2</sub>.

17. A kringle 5 peptide fragment which has substantial sequence homology to a plasminogen fragment selected from human, murine, bovine, Rhesus monkey and porcine plasminogen.

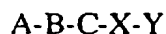
18. A kringle 5 peptide fragment or fusion protein wherein the kringle 5 peptide fragment or kringle 5 fusion protein has a substantial sequence homology to human plasminogen.

19. A method of treating a disease in a patient in need of antiangiogenesis therapy comprising administering to a human or animal a therapeutically effective amount of a mammalian kringle 5 peptide fragment or kringle 5 fusion protein.

20. The method of Claim 19 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is selected from the group consisting of a human, murine, bovine, Rhesus monkey and porcine kringle 5 peptide fragment or fusion protein.

21. The method of Claim 20 wherein said kringle 5 peptide fragment or kringle 5 fusion protein is a human kringle 5 peptide fragment or kringle 5 fusion protein.

22. The method of Claim 19 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula



(I)

5 or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl, 3-l-tyrosyl or phenylalanyl;

R<sup>4</sup> is aspartyl; and

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1 and homologues or analogues thereof.

23. The method of Claim 22 wherein said mammalian kringle 5 fragment or kringle 5 fusion protein is said compound wherein A and Y are as defined therein and B-C-X is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;
- (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;
- (g) the sequence from amino acid positions 449-546 of SEQ ID NO:1;
- (h) the sequence from amino acid positions 454-546 of SEQ ID NO:1;
- (i) the sequence from amino acid positions 525-535 of SEQ ID NO:1;
- (j) the sequence from amino acid positions 529-535 of SEQ ID NO:1; and
- (k) the sequence from amino acid positions 530-535 of SEQ ID NO:1.

24. The method of Claim 22 said compound is said mammalian kringle 5 fragment wherein X is absent, A and Y are as defined therein and B-C is the sequence from amino acid positions 529-534 of SEQ ID NO:1.

25. The method of Claim 22 wherein said compound is said mammalian kringle 5 fragment wherein X and B are absent, and A, C and Y are as defined therein.

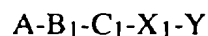
26. The method of Claim 23 or 24 or 25 wherein A is N-Ac and Y is -NH<sub>2</sub>.

27. The method of Claim 19 wherein said disease is selected from the group consisting of cancer, arthritis, macular degeneration and diabetic retinopathy.

28. The method of Claim 27 wherein said disease is cancer.

29. The method of Claim 28 wherein said disease is selected from primary and metastatic solid tumors, carcinomas, sarcomas, lymphomas, psoriasis and hemangiomas.

30. The method of Claim 19 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula



(II)

5 or a pharmaceutically acceptable salt, ester or prodrug thereof, wherein

A is absent or a nitrogen protecting group;

Y is absent or a carboxylic acid protecting group;

10 B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

15 X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID NO:1 and homologues or analogues thereof.

31. The method of Claim 30 wherein said compound is said mammalian kringle 5 peptide fragment wherein B<sub>1</sub> and X<sub>1</sub> are absent, A, C<sub>1</sub> and Y are as defined therein.

32. A composition comprising an isolated single- or double-stranded polynucleotide sequence that encodes a kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

33. The composition of Claim 32 wherein said polynucleotide sequence is a DNA sequence.

34. The composition of Claim 33 wherein said DNA sequence encodes an amino acid sequence selected from the group consisting of

(a) the sequence from amino acid positions 443-543 of SEQ ID NO:1;

(b) the sequence from amino acid positions 449-543 of SEQ ID NO:1;



- 5           (c)     the sequence from amino acid positions 454-543 of SEQ ID NO:1; and  
          (d)     the sequence from amino acid positions 355-543 of SEQ ID NO:1.

35.     The composition of Claim 33 wherein said polynucleotide sequence encodes an amino acid sequence selected from the group consisting of SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, and SEQ ID NO:37.

36.     A composition comprising a kringle 5 peptide fragment or kringle 5 fusion protein and a pharmaceutically acceptable excipient.

37.     A method comprising implanting into a human or non-human animal a cell containing a vector, wherein said vector contains a DNA sequence encoding a kringle 5 peptide fragment or kringle 5 fusion protein and wherein said vector is capable of expressing said kringle 5 peptide fragment or kringle 5 fusion protein when present in said  
5     cell.

38.     A method of making a kringle 5 peptide fragment comprising the steps of:  
          (a) exposing a mammalian plasminogen to elastase at a ratio of about 1:100 to about 1:300 to form a mixture of said plasminogen and said elastase;  
          (b) incubating said mixture; and  
5       (c) isolating said kringle 5 from said mixture.

39.     An isolated single- or double-stranded polynucleotide which encodes a mammalian kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

40.     The polynucleotide of Claim 39 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein encoded by said polynucleotide is selected from the group consisting of human, Rhesus monkey, bovine, murine, and porcine kringle 5 peptide fragment or fusion protein.

41.     The polynucleotide of Claim 40 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a human kringle 5 peptide fragment or kringle 5 fusion protein.

42.     The polynucleotide of Claim 39 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound having the formula B-C-X or B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub> wherein

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl or phenylalanyl; and

R<sup>4</sup> is aspartyl;

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1;

B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID NO:1 and complements thereof.

43. The polynucleotide of Claim 42 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein B is present and C and X are as defined therein.

44. The polynucleotide of Claim 42 wherein said a mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein X is present and B and C are as defined therein.

45. The polynucleotide of Claim 42 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a fragment wherein B and X are present and C is as defined therein.

46. The polynucleotide of Claim 39 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;

- 10 (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;  
(f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;  
(g) the sequence from amino acid positions 449-546 of SEQ ID NO:1; and  
(h) the sequence from amino acid positions 454-546 of SEQ ID NO:1.

47. The polynucleotide of Claim 42 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a fragment wherein X is absent and B and C are as defined therein.

48. The polynucleotide of Claim 39 which is a DNA molecule.

49. The polynucleotide of Claim 39 which is an RNA molecule.

50. A vector comprising a polynucleotide which encodes a mammalian kringle 5 peptide fragment or kringle 5 fusion protein having angiogenesis inhibiting activity.

51. The vector of Claim 50 which is an expression vector.

52. The vector of Claim 51 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein encoded by said polynucleotide is a compound having the formula B-C-X or B<sub>1</sub>-C<sub>1</sub>-X<sub>1</sub> wherein

B is absent or is from 1 to about 197 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 530 of SEQ ID NO:1;

C is R<sup>1</sup>-R<sup>2</sup>-R<sup>3</sup>-R<sup>4</sup> wherein

R<sup>1</sup> is lysyl;

R<sup>2</sup> is leucyl or arginyl;

R<sup>3</sup> is tyrosyl or phenylalanyl; and

R<sup>4</sup> is aspartyl;

X is absent or is from 1 to about 12 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 535 to about amino acid position 546 of SEQ ID NO:1;

B<sub>1</sub> is absent or is from 1 to about 176 naturally-occurring amino acid residues corresponding to the sequence from about amino acid position 334 to amino acid position 513 of SEQ ID NO:1;

C<sub>1</sub> is the sequence from amino acid position 514 to amino acid position 523 of SEQ ID NO:1; and

X<sub>1</sub> is absent or is from 1 to about 10 naturally-occurring amino acid residues corresponding to the sequence from amino acid position 524 to amino acid position 533 of SEQ ID NO:1 and complements thereof.  
and complements thereof.

53. The vector of Claim 52 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein B and X are present and C is as defined therein.

54. The vector of Claim 52 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is a compound wherein X is absent and B and C are as defined therein.

55. The vector of Claim 52 wherein said mammalian kringle 5 peptide fragment or kringle 5 fusion protein is selected from the group consisting of

- (a) the sequence from amino acid positions 355-543 of SEQ ID NO:1;
- (b) the sequence from amino acid positions 355-546 of SEQ ID NO:1;
- 5 (c) the sequence from amino acid positions 443-543 of SEQ ID NO:1;
- (d) the sequence from amino acid positions 449-543 of SEQ ID NO:1;
- (e) the sequence from amino acid positions 454-543 of SEQ ID NO:1;
- (f) the sequence from amino acid positions 443-546 of SEQ ID NO:1;
- (g) the sequence from amino acid positions 449-546 of SEQ ID NO:1; and
- 10 (h) the sequence from amino acid positions 454-546 of SEQ ID NO:1.

56. The vector of Claim 52 selected from the group consisting of pHil-D8, pET32a, pGEX-4T-2, Up-ET, UpET-Ubi, and pCYB3.

57. The vector of Claim 52 further comprising a host cell transformed with said vector.

58. The vector of Claim 57 wherein said host cell is a eukaryotic cell.

59. The vector of Claim 58 wherein said eukaryotic cell is *Pichia pastoris*.

60. The vector of Claim 57 wherein said host cell is a prokaryotic cell which is *E. coli*.

61. A method for making a soluble kringle 5 peptide fragment or kringle 5 fusion protein comprising the steps of:

- (a) isolating a polynucleotide which encodes said kringle 5 peptide fragment;

- 5 (b) cloning said polynucleotide into an expression vector;  
(c) transforming said vector into a suitable host cell; and  
(d) growing said host cell under conditions suitable for the expression of said soluble kringle 5 peptide fragment or kringle 5 fusion protein.

62. A compound selected from the group consisting of

- (a) A-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-Y;
- (b) A-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-Y;
- (c) A-Pro-Glu-Lys-Arg-Tyr-Asp-Tyr-Y; and
- (d) A-Gln-Asp-Trp-Ala-Ala-Gln-Glu-Pro-His-Arg-His-Ser-Ile-Phe-Thr-Pro-Glu-Thr-Pro-Glu-Thr-Asn-Pro-Arg-Ala-Gly-Leu-Glu-Lys-Asn-Tyr-Y.

1/10

FIG. 1(a) (SEQ ID NO:1)

GLU	PRO	LEU	ASP	ASP	TYR	VAL	ASN	THR	GLN	GLY	ALA	SER	LEU	PHE	1	5	10	15
SER	VAL	THR	LYS	LYS	GLN	LEU	GLY	ALA	GLY	SER	ILE	GLU	GLU	CYS	20	25	30	
ALA	ALA	LYS	CYS	GLU	GLU	ASP	GLU	GLU	PHE	THR	CYS	ARG	ALA	PHE	35	40	45	
GLN	TYR	HIS	SER	LYS	GLU	GLN	GLN	CYS	VAL	ILE	MET	ALA	GLU	ASN	50	55	60	
ARG	LYS	SER	SER	ILE	ILE	ILE	ARG	MET	ARG	ASP	VAL	VAL	LEU	PHE	65	70	75	
GLU	LYS	LYS	VAL	TYR	LEU	SER	GLU	CYS	LYS	THR	GLY	ASN	GLY	LYS	80	85	90	
ASN	TYR	ARG	GLY	THR	MET	SER	LYS	THR	LYS	ASN	GLY	ILE	THR	CYS	95	100	105	
GLN	LYS	TRP	SER	SER	THR	SER	PRO	HIS	ARG	PRO	ARG	PHE	SER	PRO	110	115	120	
ALA	THR	HIS	PRO	SER	GLU	GLY	LEU	GLU	GLU	ASN	TYR	CYS	ARG	ASN	125	130	135	
PRO	ASP	ASN	ASP	PRO	GLN	GLY	PRO	TRP	CYS	TYR	THR	THR	ASP	PRO	140	145	150	
GLU	LYS	ARG	TYR	ASP	TYR	CYS	ASP	ILE	LEU	GLU	CYS	GLU	GLU	GLU	155	160	165	
CYS	MET	HIS	CYS	SER	GLY	GLU	ASN	TYR	ASP	GLY	LYS	ILE	SER	LYS	170	175	180	
THR	MET	SER	GLY	LEU	GLU	CYS	GLN	ALA	TRP	ASP	SER	GLN	SER	PRO	185	190	195	
HIS	ALA	HIS	GLY	TYR	ILE	PRO	SER	LYS	PHE	PRO	ASN	LYS	ASN	LEU	200	205	210	
LYS	LYS	ASN	TYR	CYS	ARG	ASN	PRO	ASP	ARG	GLU	LEU	ARG	PRO	TRP	215	220	225	
CYS	PHE	THR	THR	ASP	PRO	ASN	LYS	ARG	TRP	GLU	LEU	CYS	ASP	ILE	230	235	240	
PRO	ARG	CYS	THR	THR	PRO	PRO	PRO	SER	SER	GLY	PRO	THR	TYR	GLN	245	250	255	
CYS	LEU	LYS	GLY	THR	GLY	GLU	ASN	TYR	ARG	GLY	ASN	VAL	ALA	VAL	260	265	270	

SUBSTITUTE SHEET (RULE 26)